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THE SWIMBLADDER GAS CONTENT OF SOME FRESHWATER FISH WITH PARTICULAR REFERENCE TO THE PHYSOSTOMES[1]

BY RICHARD L. SAUNDERS

Abstract

Swimbladder gas samples were analyzed from 10 species of fish caught at various depths extending from the surface to 486 ft. in Lake Huron and adjoining waters. The physoclists studied, yellow perch and burbot, had gas compositions which agreed with previous findings. From progressively greater depths, these displayed decreasing nitrogen percentages and increasing oxygen percentages. The physostomes, lake trout, lake whitefish, shallowwater cisco, deepwater cisco, bloater, American smelt, white sucker, and longnose sucker, displayed strikingly high percentages of nitrogen and correspondingly low percentages of oxygen at all depths. Carbon dioxide was found only in traces in both groups. Results of flotation pressures determined for American smelt, shallowwater cisco, and bloater indicated that most specimens were probably buoyant at depths of capture and that results of gas analyses were indicative of the gas compositions at depths of capture. Both physostomes and physoclists from the greatest depths of capture displayed swimbladder nitrogen pressures in excess of 0.8 of an atmosphere, the partial pressure of dissolved nitrogen in most natural waters. The excess was slight in physoclists but in physostomes it was nearly equal to the total (hydrostatic plus atmospheric) pressure.

Introduction

The gases found in swimbladders of fish are rarely in the same proportions as are found in the atmosphere. This fact has stimulated many workers to investigate the function of gaseous exchange in the swimbladder. However, one major division of fishes, as based on the anatomy of the swimbladder, has been almost entirely neglected in these considerations. This division, the physostomes, embraces those fishes having pneumatic ducts connecting the swimbladder to the gut. The purpose of the present investigation was to obtain knowledge of swimbladder gas in physostomes.

The physostomes comprise a major division of the soft-rayed fish and include among others the following families: Salmonidae, Coregonidae, Osmeridae, Catostomidae, and Cyprinidae. Fish in which there is no pneumatic duct connecting the swimbladder to the gut are called physoclists. These are typically the spiny-rayed fish, but this group includes also some families having soft-rayed fins. It is with this latter group that past swimbladder studies have been mainly concerned.

[1] *Manuscript received June 3, 1953.*
Contribution from the Ontario Fisheries Research Laboratory, Department of Zoology, University of Toronto, Toronto, Ontario.

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TABLE I

Results of swimbladder gas analyses

Results are arranged in groups of specimens taken from depths corresponding to half atmosphere intervals. Figures are mean percentage compositions of carbon dioxide, oxygen, and nitrogen from all fish from specified depth intervals. The first eight species listed are physostomes, the remaining two are physoclists.

Species	Depth range, ft.	Carbon dioxide	Oxygen	Standard error or range	Nitrogen	Standard error or range	Number of specimens
Lake trout, *Salvelinus namaycush* (Walbaum)	0 – 16.5	0.4	0.3	0 – 1.05	99.3	98.6– 99.8	7
	16.5– 33.0	1.9	1.2	—	96.9	—	1
	66.0– 82.5	0.3	0.6	0 – 1.31	99.2	98.2–100.0	15
	99.0–115.5	0.1	0.7	0.6– 0.7	99.2	99.0– 99.4	2
	115.5–132.0	0.2	0.1	0 – 0.6	99.7	99.4–100.0	6
Lake whitefish, *Coregonus clupeaformis* (Mitchill)	0 – 16.5	1.5	2.8	0 –18.8	95.7	0.3	209
	16.5– 33.0	1.4	4.9	0 –42.3	94.7	0.4	305
	33.0– 49.5	1.2	3.8	0 –26.4	95.2	0.5	129
	49.5– 66.0	0.5	0.0	—	99.5	—	1
	66.0– 82.5	0.9	1.3	0 – 8.3	97.8	0.4	41
	115.5–132.0	0.6	1.9	0 – 8.1	97.5	0.7	15
	148.5–165.0	0.2	0.0	—	99.8	—	1
Shallowwater cisco, *Leucichthys artedi* (LeSueur)	0 – 16.5	1.6	10.7	0 –24.7	87.7	1.9	53
	16.5– 33.0	1.3	15.8	0 –43.5	82.9	55.4– 98.2	6
	49.5– 66.0	1.3	12.4	6.4–17.1	86.4	80.9– 92.8	8
	66.0– 82.5	0.9	11.9	3.0–16.7	87.2	82.7– 95.8	9
	82.5– 99.0	0.8	11.1	0.7–33.7	88.1	65.5– 98.6	12
	115.5–132.0	1.1	10.9	5.5–18.8	88.0	0.9	15
	132.0–148.5	0.7	17.2	8.4–39.4	82.2	59.4– 91.3	12
	148.5–165.0	0.7	12.4	0.1–47.5	86.8	1.6	38
	231.0–247.5	0.4	4.2	1.1– 8.4	95.4	91.2– 98.5	3*
	264.0–280.5	0.5	6.8	0 –10.6	92.7	88.6– 99.7	3*
	346.5–363.0	0.7	7.8	—	91.5	—	1*
	478.5–495.0	0.9	12.4	—	86.7	—	1*
Bloater, *Leucichthys hoyi* (Gill)	66.0– 82.5	0.9	7.9	0.5–15.2	91.2	84.6– 98.9	12
	115.5–132.0	0.7	9.9	0.2–38.3	89.4	1.6	24
	148.5–165.0	0.7	5.5	0 –35.8	93.9	1.4	35

	231.0–247.5	0.4	1.8	0 –10.8	97.8	0.5	28
	264.0–280.5	0.2	0.8	0 – 5.7	99.0	94.0–100.0	8
	346.5–363.0	0.5	1.0	0 – 5.4	98.5	0.3	18
	478.5–495.0	0.4	1.7	0 – 4.7	97.9	94.7– 99.7	6
Deepwater cisco, *Leucichthys johannae* (Wagner)	132.0–148.5	0.9	0.7	—	98.4	—	1*
	148.5–165.0	0.6	5.5	0 – 9.1	93.9	90.1– 99.8	4*
	231.0–247.5	0.5	1.9	0 – 6.2	97.6	94.5– 99.9	9
	264.0–280.5	0.2	1.0	0 – 6.0	98.9	93.4–100.0	8
	346.5–363.0	0.3	1.9	0 –10.8	97.8	0.5	26
	478.5–495.0	0.5	0.4	0 – 1.9	99.1	0.3	17
American smelt, *Osmerus mordax* (Mitchill)	0 – 16.5	1.0	11.2	0.3– 1.8	87.9	82.4– 94.3	12
	49.5– 66.0	0.8	11.7	—	87.5	—	1
	66.0– 82.5	0.6	10.9	5.5–14.5	88.5	0.7	18
	99.0–115.5	0.5	9.5	7.5–12.5	90.0	87.3– 91.8	5
	115.5–132.0	0.4	11.9	7.0–17.8	87.7	0.7	20
	132.0–148.5	0.5	8.6	7.1–13.0	90.9	86.6– 92.2	4
	148.5–165.0	0.4	5.3	0.3–15.2	94.3	0.9	22
White sucker, *Catostomus commersoni* (Lacépède)	0 – 16.5	2.0	6.9	2.7–12.9	91.1	88.3– 95.7	13
	16.5– 33.0	1.9	8.3	3.8–42.0	89.8	1.0	41
	33.0– 49.5	1.8	5.6	0 –12.3	92.6	0.6	32
	66.0– 82.5	1.7	4.7	1.2–12.1	93.6	86.7– 97.3	7
Longnose sucker, *Catostomus catostomus* (Forster)	16.5– 33.0	2.1	5.8	1.4–11.3	92.0	85.5– 97.0	12
	33.0– 49.5	1.7	4.3	—	93.1	—	1
	66.0– 82.5	1.6	7.5	2.0–10.1	90.9	87.0– 96.4	14
Burbot, *Lota lota* (Linnaeus)	16.5– 33.0	1.4	34.4	1.8	64.2	1.9	17
	49.5– 66.0	0.8	65.0	—	34.2	—	1
	66.0– 82.5	0.9	59.8	45.3–72.5	39.3	26.9– 53.8	9
	99.0–115.5	0.5	74.2	73.3–75.1	25.3	24.6– 26.1	2
	132.0–148.5	0.6	76.5	—	22.9	—	1
	148.5–165.0	1.1	81.3	—	17.6	—	1
	346.5–363.0	1.1	87.8	—	11.1	—	1
Yellow perch, *Perca flavescens* (Mitchill)	0 – 16.5	1.3	31.9	1.1	66.8	1.2	42
	16.5– 33.0	1.0	36.9	—	62.1	—	1
	33.0– 49.5	1.5	45.1	1.7	53.5	1.7	24
	49.5– 66.0	2.1	48.4	22.1–64.5	49.5	33.7– 77.3	13

* *These data were not used in constructing Fig. 1.*

volume of water displaced by the collected gas was recorded and the volume of gas at atmospheric pressure was calculated from these data. No correction was made for temperature.

After all gas had been removed and its volume measured, each fish was carefully weighed under water in the manner described by Tester (20), that is, by suspending it on a wire from the arm of a balance. The gas volume divided by the weight in water gave a value which is referred to as the flotation pressure. Flotation pressures were expressed in atmospheres.

Results

Carbon Dioxide

Results of gas analyses for eight species of physostomes and two species of physoclists are summarized in Table I. Analyses of swimbladder gas from both physostomes and physoclists revealed only slight amounts of carbon dioxide in the majority of cases. These slight amounts of carbon dioxide should be considered as traces because the method of analysis does not allow accurate determination of such small quantities of gas. Furthermore, there is no measurable relation between the percentage of carbon dioxide and depth such as has been found for oxygen in the physoclists and nitrogen in both physostomes and physoclists. In consequence, the carbon dioxide content will not be discussed further.

Nitrogen

The most striking find was that the relationship between nitrogen content and depth is opposite in physostomes and physoclists. Both groups displayed similar high percentages of swimbladder nitrogen in shallow water, but with increasing depths of capture there was a marked divergence in the gas composition. The physoclists, yellow perch and burbot, displayed the well established pattern of reduction in nitrogen percentage with depth increases. In the physostomous group, on the other hand, no species investigated had reduced percentages of nitrogen in deeper water. Lake trout, shallowwater cisco, and longnose sucker maintained their initially high nitrogen percentages, and the five remaining species of physostomes displayed increases in nitrogen percentages with depth until the bladder contained nearly pure nitrogen.

The differences in nitrogen content of physostomes and physoclists are more apparent when these percentages have been converted to nitrogen pressures and plotted in relation to total pressure in atmospheres (Fig. 1). Although swimbladder nitrogen pressure increases with depth in both physostomes and physoclists, the trend is essentially different in these two groups. Physoclists are characterized by a minor, although definite, increase above 0.8 of an atmosphere* in swimbladder nitrogen pressure as depth increases; physostomes display a major increase in nitrogen pressure throughout their depth ranges.

** Nitrogen is present at a pressure of 0.8 of an atmosphere at all depths in lakes and in the sea in all but exceptional circumstances.*

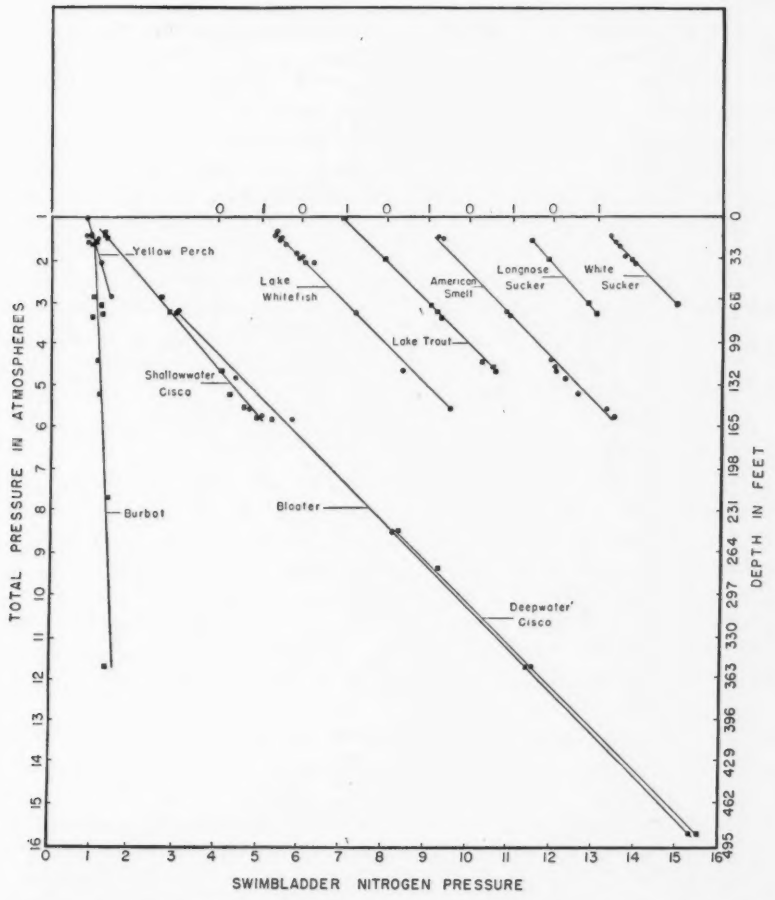

FIG. 1. Swimbladder nitrogen pressure in relation to total pressure (hydrostatic plus atmospheric). Each point represents a mean of nitrogen pressures for the total number of each species of fish caught at that depth. Nitrogen pressure is obtained by multiplying percentage nitrogen by total pressure in atmospheres at depth of capture. The curves for the five species indicated at the right were removed from the area occupied by the curves for shallowwater cisco, bloater, and deepwater cisco to avoid confusion. Note the respective origins of the various nitrogen pressure scales given along the surface datum line for displaced curves.

One early worker has reported finding high percentages of nitrogen in freshwater fish from deep water. Hüfner (8) found high nitrogen values for swimbladder gas of the whitefish or kilch, *Coregonus acronius*, from a depth of 60-80 meters in Lake Constance in Europe. In six out of nine specimens he found that swimbladders contained over 99% nitrogen.

Oxygen

Analyses for oxygen show that there is essentially a complementary relationship between oxygen and nitrogen percentages in swimbladders of physostomes and physoclists. The obvious characteristic of oxygen composition in physoclistous swimbladders is a steady increase with depth as illustrated by the data for yellow perch and burbot. This increase of oxygen with depth has been noted previously by many workers in the field from Biot (1) to Scholander *et al.* (18) and Scholander and van Dam (19). In the physostomes, on the other hand, there is much less oxygen than there is in the physoclists. In the lake trout, lake whitefish, and deepwater cisco, swimbladder gases contain only small percentages of oxygen. Swimbladders of shallowwater cisco, bloater, American smelt, white sucker, and longnose sucker contain oxygen in slightly greater proportions than the other physostomes investigated. No relationship between oxygen pressure and hydrostatic pressure could be established for any of the physostomes.

Flotation Pressure

Flotation pressures were determined for three of the physostomes, American smelt, shallowwater cisco, and bloater, and the results for the first and third species are summarized in Figs. 2 and 3 respectively. One reason for determining flotation pressures of physostomes was to ascertain whether fish were in hydrostatic equilibrium at depths of capture. Another reason was to find out whether observed percentages of swimbladder gas were indicative of the gas compositions at depths where fish were caught. Results obtained for the three species investigated indicate that many of the specimens

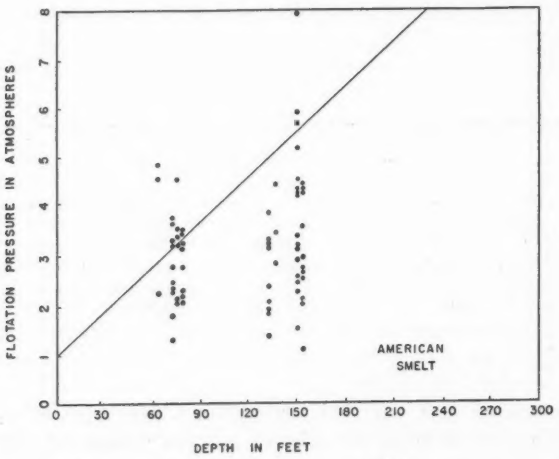

FIG. 2. Flotation pressure of American smelt in relation to depth of capture. The points given represent flotation pressures of individual fish, and the drawn line represents flotation pressures at which fish would be buoyant at particular depths.

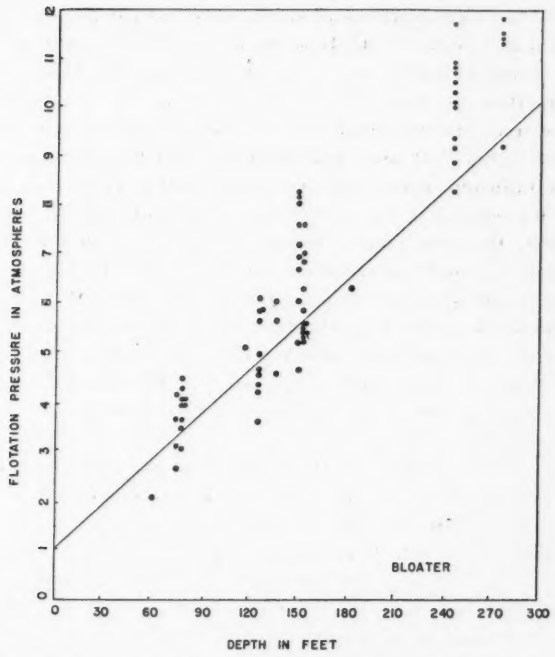

FIG. 3. Flotation pressure of bloater in relation to depth of capture. The points given represent flotation pressures of individual fish, and the drawn line represents flotation pressures at which fish would be buoyant at particular depths.

were in hydrostatic equilibrium and that observed percentages of swimbladder gas were indicative of the gas compositions at depths where these fish were caught.

In general, in the three species investigated, fish from comparatively shallow water had flotation pressures which were sufficient for the fish to have been in hydrostatic equilibrium at the depths of capture. From greater depths, where there was a greater reduction in pressure resulting from lifting the gill nets, American smelt and shallowwater cisco had flotation pressures below those necessary for buoyancy. This condition of low flotation pressures is illustrated by the data for American smelt shown in Fig. 2. Bloaters (Fig. 3) had flotation pressures sufficient for buoyancy at all depths for which determinations were made.

Many specimens of American smelt and shallowwater cisco had flotation pressures which were too low for these fish to have been in hydrostatic equilibrium at depths of capture. This phenomenon is quite easily explained. In smelt particularly, there is a tendency for gas to escape from the swimbladder as the net is lifted and hydrostatic pressure is reduced.* It is possible to

** The author has observed lake whitefish releasing gas bubbles as they were forced to come to the surface during the lifting of pound nets.*

force gas out of the swimbladders of smelt with a slight pressure on the side of the fish. On the contrary, the body wall and swimbladder of the bloater would break before a bubble of gas could be squeezed from the bladder. Reasonable flotation pressures for bloaters, smelt, and shallowwater cisco from shallow water, and the demonstrated tendency for smelt to lose gas help to reinforce the belief that most specimens studied had enough gas to be in hydrostatic equilibrium before they were subjected to reductions in pressure.

Jones (12) has shown in his studies of vertical movements of the perch, *Perca fluviatilis*, that this physoclistous species . . . "can only swim freely above their plane of equilibrium within a narrow zone whose height is equivalent to a 16 per cent reduction in total pressure to which they are adapted." Of the bloaters in the present study, some individuals contained gas volumes which would have been sufficient to make them buoyant at depths up to 33% greater than those at which they were caught. These fish containing more than the volume of gas necessary for buoyancy would have to expend energy to resist upward movement and consequent greater reductions in pressure. The differences between observed flotation pressures and those expected in light of Jones's work are greater than can be explained by error in measurement.

High flotation pressures could be explained in the following way. Fish having seemingly too high flotation pressures may have recently moved inshore from deeper water. In fish which had just moved from deep to comparatively shallow water, the swimbladder would have a gas volume in excess of that necessary for hydrostatic equilibrium at the new, lesser depth. This condition of excess gas is particularly well illustrated by the graph for bloater. Some species of fish are thought to move into shallow water at night to feed. It is possible that these fish displaying high flotation pressures had just recently moved in from deeper water and had not adjusted their swimbladder volumes to correspond to the shallower water before being caught.

Discussion

Physoclists

Physoclists studied in the present investigation displayed swimbladder gas compositions similar to those of physoclists studied by other workers. In general, the results presented here agree with the findings described by: Biot (1); Richard (15); Schloesing and Richard (17); Hall (7); Rostorfer (16); Scholander *et al.* (18); and Scholander and van Dam (19). It has been found by these workers and in the present investigation that the percentage of nitrogen in physoclistous swimbladders decreases with depth. Conversely, oxygen percentages steadily increase with depth and are essentially complements of corresponding nitrogen percentages.

One detail of swimbladder performance overlooked until recently is the increase in nitrogen pressure concurrent with depth increase. Superficially, physoclist swimbladder nitrogen appears to decrease with depth. Only when percentages have been converted to nitrogen pressures is the true picture revealed. Scholander *et al.* (18) and Scholander and van Dam (19) were the

first to recognize the true relation between nitrogen content of swimbladders and depth. From their investigations on the New England coast and Bimini in the Bahamas, Scholander and van Dam reported finding swimbladder nitrogen at pressures in excess of 0.8 of an atmosphere for marine physoclists taken at depths from zero to nearly 1000 meters. In the present investigation the data for yellow perch and burbot confirmed these findings for freshwater physoclists. In Lake Huron, as in the Atlantic Ocean, it may be taken that nitrogen is present at all depths at 0.8 of an atmosphere. The problem of accounting for the excess pressure of nitrogen, a physiologically inert gas, over the pressure of nitrogen dissolved in water is being attacked by Scholander, and the present author has nothing to add except to offer these data for freshwater physoclists in confirmation of swimbladder findings in the sea.

Physostomes

If the minor excesses of nitrogen in physoclists present a problem, the major excesses of nitrogen in swimbladders of physostomes present a still greater one. The general misconception that all physostomes fill their swimbladders by gulping air has led many investigators to dismiss the problem of gaseous exchange in the latter group and focus their attention on the physoclists in which exchange must be by way of the blood. The present investigation is mainly concerned with the physostomes because there is little known of the swimbladder gases in this group.

Current Theories of Swimbladder Gaseous Exchange in Physostomes

Current theories attempting to explain gaseous exchange in physostomous swimbladders are not in accord with the findings reported here. The theory that gulping air at the surface suffices in all cases for filling swimbladders of those fish having pneumatic ducts can be dismissed for reasons pointed out earlier (page 548). Although air-gulping by fish has been observed by many investigators (Rauther (14); Evans and Damant (5); Jacobs (10, 11); von Ledebur (13); and Black (2)), this means of filling swimbladders is inadequate in many species and a seeming impossibility in others.

A second theory is that gaseous exchange is basically the same in both physostomes and physoclists, although proceeding more slowly in physostomes (von Ledebur (13)). Because of the profound differences in the nitrogen–oxygen relationships in the two groups, the present concept of swimbladder performance based on a study of the physoclists must be abandoned when we undertake a study of physostomes, at least for those physostomes without gas glands. Jacobs (10) has studied gaseous exchange in swimbladders of a series of physostomes belonging to the three families, Salmonidae, Esocidae, and Cyprinidae. Although the fish investigated, *Hucho*, *Salmo*, *Esox*, *Phoxinus*, and *Cyprinus* were able to fill experimentally emptied swimbladders by gulping air at the surface, there was considerable variation in the speed and ability of these fish in refilling their swimbladders when they were barred access to air

by a wire screen. Of the species investigated, pike were best able to fill their swimbladders without gulping air. They required from five to 17 days to complete the filling under the conditions of the experiment.* Pike resemble the physoclists in their possession of gas glands. Cyprinids could fill their swimbladders under the conditions of the experiment, but still more slowly than pike. The salmonoids were unable to fill their swimbladders at all when they were prevented from gulping air. Jacobs' results demonstrate that although there may be some gaseous secretion in swimbladders of certain physostomes, it is a very slow process where it does occur, and it is not an adequate mechanism for swimbladder gaseous regulation in all physostomes.

In light of the recent ingenious experiments of Copeland (4), it is possible that Jacobs' (10) experiments were imperfect. It is necessary to carry Jacobs' experiments one step further and to consider the reflex which controls swimbladder activity. Copeland has shown experimentally for the mummichog, *Fundulus heteroclitus* (a physoclist), that swimming up or down provides the stimulus for the reflex action of swimbladder filling. ". . . The receptors of the reflexes controlling activity of the swimbladder are located in the musculature of the pectoral fins, since these structures are primarily used in achieving a new orientation in the vertical plane." In Jacobs' (10) experiments to determine the ability of fish to refill swimbladders that have been experimentally emptied, there was apparently no provision made to cause fish to swim and thus stimulate bladder filling. It is possible that fish with emptied swimbladders would sink to the bottom where they would remain inactive during the experiment. If the reflex for swimbladder filling is similar in physostomes and physoclists, then it is necessary to stimulate experimental fish to swim up or down before a true picture of the comparative mechanisms of gaseous exchange in these two groups can be had.

A third mechanism for the deposition of gas in swimbladders of physostomes was suggested by Irving and Grinnell (9). Taking brook trout as an example, Irving and Grinnell suggested that the effect of carbon dioxide on fish blood, that is, of greatly diminishing the combining power of blood with oxygen (the Bohr effect), would be sufficient to elevate greatly the pressure of oxygen and make it available for the swimbladder. This suggestion must be abandoned as a possible mechanism of gaseous exchange in salmonoid swimbladders because it is not consistent with the findings in the present investigation in which no physostome studied had swimbladder gas which was predominantly oxygen. In fact, oxygen was almost entirely lacking in swimbladders of some physostomes, and in others, it decreased from initially low percentages as depth of capture was increased.

At present the problem of gaseous regulation in swimbladders of physostomes is as far from solution as ever. Three mechanisms for gaseous exchange

** Of the physostomes studied in the present investigation two catostomids, the white sucker and the longnose sucker, are the most highly evolved. Catostomids and cyprinids belong to the order Ostariophysi, in which there is some deposition of gases in the swimbladder as Jacobs has demonstrated with minnows. The remaining six physostomous species in the present investigation belong to the more primitive order, Isospondyli, as do Salmo and Hucho, two species which Jacobs found incapable of augmenting swimbladder volumes when access to the air was precluded.*

in swimbladders of this group have been given with little or no consideration for the biology of physostomes. First of all, it should have been obvious from observations of the vertical movements of many physostomes that the first mentioned and most widely accepted mechanism for swimbladder gaseous exchange, that is, gulping air at the surface, would be restricted in applicability. The results of gas analyses presented here give sufficient evidence to invalidate the second theory, at least as it is understood at present. The differences in nitrogen–oxygen relationships in physostomes and physoclists are obviously associated with morphological differences in swimbladders of these two groups. The third-mentioned gas regulatory mechanism, as suggested by Irving and Grinnell, must be dismissed because it is not consistent with the present findings.

Summary

1. Carbon dioxide is present in traces in swimbladders of both physostomes and physoclists.

2. Nitrogen content is an essential point of difference between swimbladder gases of physostomes and physoclists. Nitrogen percentages are initially high in both groups. In physostomes at increasing depths this gas either remains at the initially high levels or increases to nearly 100%. Physoclists have decreasing percentages of nitrogen at increasing depths.

In both groups, specimens from the greatest depths of capture displayed swimbladder nitrogen at pressures in excess of 0.8 of an atmosphere, the partial pressure of nitrogen dissolved in most natural waters. The excess is slight in physoclists, but at maximum depths of capture for eight physostomes studied, nitrogen pressures were nearly equal to the total pressure in atmospheres. Therefore nitrogen, and not carbon dioxide or oxygen, is of primary importance in maintaining hydrostatic equilibrium in physostomes.

3. Oxygen percentages and pressures are essentially complementary to those of nitrogen in both groups. In physoclists, oxygen plays a greater part in maintaining hydrostatic equilibrium than does nitrogen. Oxygen pressures increase with depth in this group but not to the same extent as nitrogen pressures do in physostomous swimbladders.

4. Flotation pressures were determined for three physostomous species, American smelt, shallowwater cisco, and bloater. Flotation pressures for the bloater were sufficient for hydrostatic equilibrium at all depths for which determinations were made. From comparatively shallow stations, American smelt and shallowwater cisco contained enough gas to have been buoyant, but from greater depths, reductions in pressure caused by lifting the nets were apparently sufficient to force gas out through the pneumatic duct so that flotation pressures measured were too low.

5. Further studies are necessary to elucidate the mechanism, or mechanisms, of gaseous exchange in swimbladders of physostomes.

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SOMATIC CHROMOSOMES OF HIGHER DIPTERA[1]

II. DIFFERENTIATION OF SARCOPHAGID SPECIES

By J. W. Boyes[2]

Abstract

The somatic chromosome complements of nine sarcophagid species are described using methods presented in the first paper of this series. These species are *Acridiophaga aculeata, Kellymyia kellyi, Protodexia australis, P. hunteri, Pseudosarcophaga affinis, Sarcophaga aldrichi, S. cooleyi, Sarcophaga* "H", and *S. reversa. P. affinis* has 19 or 20 chromosomes whereas all the others have 12-chromosome complements most of which can be distinguished.

Introduction

It is now recognized that many species of the family Sarcophagidae are important parasites of various insects of great economic importance. The sarcophagid species mentioned in this paper are mainly parasites on nymphal and adult grasshoppers. Smith and Finlayson (8) have provided detailed descriptions and keys for the identification of immature stages of the flies parasitic on grasshoppers and have noted that "If, however, they are to be recognized as they occur within their hosts they must be distinguished in these stages". The value of analysis of chromosome complements as a means of differentiating immature stages of tachinid parasites has been previously emphasized by Boyes (1) and Boyes and Wilkes (2). In this study the somatic chromosome complements of nine species of sarcophagids have been analyzed and great differences in both the number and morphology of the chromosomes have been found. These differences are described in this paper and their value in distinguishing species at immature stages is considered.

Materials and Methods

The insects used in this study were taken in most cases from laboratory stocks reared from host collections at the Laboratory of Entomology, Belleville, Ont. Parasitic larvae in the third stadium were most suitable for study when they emerged from their hosts or as they began to pupate. Good results were also obtained using early third instar larvae reared on artificial medium. Mitotic divisions were very rare in larvae which had entered diapause.

The microscope slides were prepared and complements analyzed in all cases by the methods outlined in the first paper of this series. These methods are less satisfactory with the chromosomes of the sarcophagid species than with the tachinid species, as evidenced by slightly weaker staining. Some very good

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preparations have been obtained of all the species studied. Secondary constrictions are quite prominent in some chromosomes and are so close to the kinetochore that they may have been mistaken occasionally for it. This may result in some incorrect arm ratios but such unavoidable errors should be rare.

The species studied, with their hosts, are listed in Table I.

TABLE I

SARCOPHAGID SPECIES STUDIED, THEIR HOSTS OR OTHER SOURCES

Species	Host
Acridiophaga aculeata (Ald.)	*Melanoplus* sp. (Orthoptera: Locustidae)
Kellymyia kellyi (Ald.)	*Melanoplus* sp.
Protodexia australis Bl.	*Dichroplus* sp. (Orthoptera: Locustidae)
Protodexia hunteri (Hough)	*Melanoplus* sp.
Pseudosarcophaga affinis (Fall.)	*Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae)
Sarcophaga aldrichi Park.	*C. fumiferana* and *Malacosoma* sp. (Lepidoptera: Lasiocampidae)
Sarcophaga cooleyi (Ald.)	*C. fumiferana* (Reared on pork liver)
Sarcophaga "H"	*Melanoplus* sp.
Sarcophaga reversa Ald.	*Melanoplus* sp.

Descriptions of Somatic Complements of Sarcophagid Species

The somatic chromosome complements of eight identified species and of one other species, designated *Sarcophaga* "H" by Smith and Finlayson (8), have been drawn and analyzed. They are described in detail below.

Pseudosarcophaga affinis (Fall.)

Larvae of this species were available in large numbers from collections of spruce budworm, *Choristoneura fumiferana*. In each of 12 larvae there were nine pairs of relatively small chromosomes plus one or two tiny ones which behave normally at anaphase and will be referred to as Pair X (ten). This makes a somatic complement of 19 or 20 chromosomes for all individuals studied (though the tiny chromosomes were not seen in every cell studied).

Table II contains a summary of the results obtained which are illustrated in Text-fig. 1; Plate I, Fig. 9 is a photomicrograph of the complement. In all but Pair IX the kinetochore is terminal, or very nearly so, as seen at anaphase. However, since secondary constrictions were not clear in anaphases, it could

not be decided which end of a chromosome carries the kinetochore. For this reason kinetochores are not shown in Text-fig. 1 except for Pair IX. The "arm ratios" are calculated in relation to a secondary constriction for all chromosomes except those of Pair IX and are therefore secondary ratios. None of the metaphase complements could be analyzed as completely as desired. The analyses, though complicated, indicate that some of the pairs can be identified regularly.

TABLE II

Pseudosarcophaga affinis: ANALYSIS OF EIGHT SOMATIC COMPLEMENTS

Chromosome pair	Arm ratio, average	% of TCL**, average	% of TCL, S.D.*
I	1.76 (8)†	7.900	0.840
II	3.18 (7)	7.963	0.947
III	3.12 (7)	8.588	0.704
IV	4.55 (7)	9.213	0.360
V	6.44 (6) and 1.09 (1)	10.163	0.793
VI	1.07 (4) and 6.04 (3)	10.625	0.636
VII	1.11 (2) and 7.35 (3)	12.275	0.770
VIII	1.08 (4) and 6.99 (3)	13.525	0.925
IX	2.49 (8)	18.400	1.345
X	—	1.438	0.968

** S.D. = Standard deviation.*

*** TCL in μ = Larva 1, 39.0 Larva 3, 47.7*
" 1, 55.1 " 3, 69.3
" 2, 66.2 " 4, 84.3
" 2, 58.1 " 4, 62.9
Av. 60.3

† Numbers in parenthesis are for the number of chromosomes of Pairs VI, VII, and VIII, and of the pairs in all other cases.

Pairs I to V almost always have a single secondary constriction subdividing the chromosomes into long and short segments. Pairs I and II are nearly equal in length but Pair I has a considerably lower secondary arm ratio. Pairs II and III have similar secondary arm ratios but III is slightly longer. Pair IV is longer than III in all cells analyzed and has a higher secondary arm ratio in all cases, except one, where the secondary constrictions can be seen. Pair V is longer than IV in nearly all cells analyzed and usually has a higher secondary arm ratio. Thus, in general, with increasing length up to Pair V, the short segment constitutes a progressively smaller portion of the chromosome.

The larger pairs (VI to IX) frequently have two constrictions (both secondary in all but IX), though only one may be found in a particular chromosome. Thus Pair VI chromosomes have nearly equal segments in certain cases (average 1.07) but in other cases the segments are very unequal (average 6.04). Both constrictions were observed in one Chromosome VI in several cells. However, though two different ratios were obtained for Chromosome VII (1.11 and 7.35 as averages), two constrictions were never observed in a single chromosome. Sometimes one member had a distinct short segment whereas the adjacent homologue was divided into nearly equal segments by a secondary constriction. In two cells the homologues of Pair VII looked the same. Possibly the two types are heteromorphic sex chromosomes. Two different ratios were obtained for Pair VIII chromosomes (1.08 and 6.99 as averages) and two constrictions were noticed in one of the chromosomes of this pair in two different cells. Pair IX has a long arm containing a submedial secondary constriction and an arm ratio averaging 2.49. Chromosomes of this pair are easily recognized because of their greater length and their clearly observable kinetochores. Usually the differences in length and in the position of secondary constrictions make it possible to distinguish Pairs VI to IX.

The tiny chromosomes of Pair X are interesting and only two cells were found in which none could be located. They are clearest in the smaller division figures where, when two are present, they are closely associated. Since in half (six) of the individual larvae studied only one Chromosome X was located, whereas the other half had two, it is possible that they are the sex-determining chromosomes, but this is still uncertain.

No differences were noticed in the staining reaction of the chromosomes.

Acridiophaga aculeata (Ald.)

Thirteen somatic chromosome complements from seven larvae reared on grasshoppers, *Melanoplus* sp., at Belleville were examined in 1951. These larvae apparently consisted of five males and two females. All complements consisted of 12 chromosomes and averaged 70.4 μ with a range from 59.2 μ to 86.1 μ in TCL. The results are summarized in Table III and the complement illustrated in Text-fig. 2 and Plate I, Figs. 1 and 2.

The acrocentric sex chromosomes (Pair I) are very large and, being heterochromatic, are easily recognized. Pair II is shorter than III and has a lower arm ratio. Pairs III and IV overlap somewhat in both arm ratios and percentages of TCL. Pair V is regularly longer than IV and differs from VI in having a prominent secondary constriction near the kinetochore. Thus analysis of most figures can be accomplished without serious confusion.

Secondary constrictions divide the arms of several autosomal pairs into segments. The proximal segment of the short arm of Pair III chromosomes was 2.5 times the distal segment in three chromosomes. The long arms of 11 chromosomes of Pair V were divided to produce proximal segments averaging a third of the distal ones. Proximal segments of five short arms of

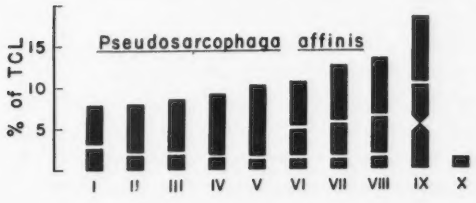


Figure 1

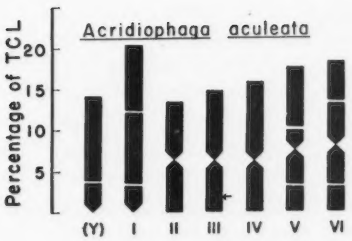


Figure 2

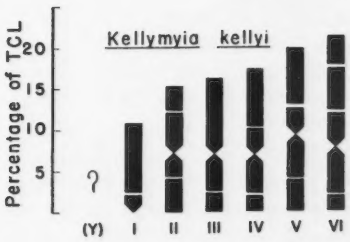


Figure 3

TEXT-FIGS. 1–3. Idiograms of somatic chromosome complements. Note that kinetochores are not indicated for most of the chromosomes of *P. affinis*. See the first paper of this series (2) for explanation of methods of constructing the idiograms.

Pair V chromosomes averaged 1.31 times the distal ones. Also the proximal to distal ratios of segments of five long arms of Pair VI averaged 1.06 and the corresponding ratio in five short arms was 1.51.

The large heterochromatic *X*-chromosomes are considerably larger than the similarly heterochromatic *Y*-chromosomes (the *Y* average corresponds to 14.1% of TCL for nine). Secondary constrictions divide the *X*-chromosome into three segments constituting, from proximal to distal end, 16.5%, 43.5%, and 40.0% of the total length. A proximal segment of the *Y*-chromosome constitutes 26.2% of its total length (see Text-fig. 2). These chromosomes are, of course, only assumed to be the sex chromosomes because one is smaller in some larvae and because of their heterochromatic nature. They appear to be, at least partly, heteropycnotic in metabolic nuclei. The existence of small heteropycnotic segments in certain autosomes is indicated also by the presence of additional darkly staining chromocenters in the metabolic nuclei.

Four somatic complements from a single larva, studied in 1947, were analyzed with results shown in Table III. The figures were small, averaging 56.6 μ, and, since they were not very well stained, they were difficult to analyze. The average ratio for Pairs II–VI was 1.33.

Kellymyia kellyi (Ald.)

Fifteen somatic complements from nine larvae reared on grasshoppers, *Melanoplus* sp., were drawn and analyzed in 1951. All had 12 chromosomes in all complements seen but another larva lacked one of the sex chromosomes (Plate I, Fig. 4). Complements analyzed ranged in TCL from 60.8 μ to 92.4 μ and averaged 74.6 μ. Results of analyses are recorded in Table III and the complement is illustrated in Text-fig. 3 and Plate I, Fig. 3.

The acrocentric sex chromosomes are smaller than the autosomes and positively heterochromatic at metaphase. Pair III has a higher average arm ratio and is longer than Pair II. Similarly Pair IV exceeds Pair III, on the average, in both respects. Pair V has a low arm ratio (1.16), thus differing from both IV (av. 1.51) and VI (av. 1.75). Also, this pair differs from IV by 2.7% of the TCL. Pairs V and VI resemble each other in length. However, chromosomes of Pair V and sometimes those of VI have a secondary constriction close to the kinetochore so that the ratios recorded may be primary or secondary. One cannot be sure which constriction is the kinetochore without careful analysis of anaphase chromosomes and this was not attempted. This difficulty does not lead to confusion because the same constriction is used consistently but until the kinetochore position is definitely known there are alternate ways of obtaining the arm ratios of these two pairs. The average of arm ratios for Pairs II–VI is 1.37.

Secondary constrictions are found in all the pairs of chromosomes but those only occasionally seen may be the product of stretching during squashing and do not merit recording. The proximal segment of the long arm of Pair II chromosomes averaged (for six) 1.45 times the distal in length and the proximal segment of the short arm averaged 0.76 of the distal for six chromosomes. Seven proximal segments of the short arm of Pair III chromosomes averaged 1.93 times the distal segments. In Pair IV long arm proximal segments averaged 0.41 of the distal for five chromosomes and short arm proximal segments averaged 2.34 of the distal for seven chromosomes. Nearly half the Pair V chromosomes had a distinct submedial constriction dividing what is presumably the long arm into segments with an average proximal to distal ratio of 0.45, whereas the corresponding "short arm" ratio was 1.32 for seven chromosomes. Similarly the "long arm" of Pair VI has a proximal to distal ratio of 2.65 for 21 chromosomes and of 0.44 for 13 chromosomes, produced by two different constrictions in these "arms". Finally, the "short arm" of Pair VI has 2.68 as the average proximal to distal ratio of segments for five chromosomes.

The two smallest chromosomes are acrocentric and heterochromatic at metaphase and are assumed to be the sex chromosomes. They sometimes differ somewhat in length but no consistent difference indicating which is the *X*-chromosome was found. All of them have been included in Pair I for convenience in analyzing. They appear to be heteropycnotic in metabolic nuclei. It seems unlikely that the single larva with one sex chromosome represents the usual condition in the male.

Three complements, from a single larva obtained in 1949, averaged 88.2 μ in length and 1.35 in average ratio of Pairs II–VI. The results of analyses were as shown in Table III. These results are fairly close to those presented above except for the secondary arm ratio of Pair I which is very high. The complement of this larva may be extreme in this respect or the larva may represent a distinct subgroup of this species. The 1949 stock was obtained in southern Saskatchewan about 70 miles from the place where the 1951 stock originated.

Protodexia australis Bl.

Several larvae of this grasshopper parasite were obtained from stocks being reared at Belleville. Six cells suitable for analysis were found in two males and one female and all contained 12 chromosomes. The TCL averaged 60.0 μ with a range from 45.8 μ to 67.6 μ. The male and female complements are shown in Plate I, Figs. 6 and 7 and results of analysis in Table III and Text-fig. 4.

The *X*-chromosomes (Pair I) are much shorter than II. Pairs II, III, and IV differ little in average length but Pair II has a lower arm ratio than the other two pairs have. Pair V must be distinguished from smaller pairs entirely by the small differences in percentage of TCL. Pair VI is obviously longer than any other pair and has an arm ratio that differs from those of other long pairs.

A subterminal secondary constriction was found in the short arm of Chromosome VI. Two *X*-chromosomes had a medial constriction which is assumed to be secondary. Each has also another constriction, which is presumably also secondary and nearer the kinetochore, but no anaphases were seen in this species. The *Y*-chromosome is very small (38.3% of *X* and corresponding to 4.0% of TCL) and lightly staining. In certain cases it has two nearly equal parts, but the constriction which separates them is probably secondary. The *X* is not noticeably heterochromatic at metaphase but there are indications of its heteropycnotic condition in metabolic nuclei.

Protodexia hunteri (Hough)

The larvae of this species were reared on grasshoppers, *Melanoplus* sp., at Belleville. The chromosome number of each of the four larvae studied (three males and one female) was 12. The complements ranged from 50.1 μ to 88.0 μ in TCL with 64.1 μ as the average. Plate I, Fig. 5 shows the somatic chromosome complement and the results of analysis of the chromosomes in 12 metaphase figures are shown in Table III and in Text-fig. 5.

The *X*-chromosomes (Pair I) averaged 7.2 as percentage of the TCL and accordingly are appreciably smaller than Pair II chromosomes which averaged 16.6 as percentage of the TCL. Pairs II and III cannot be distinguished by length differences in some cells. However, the differences in arm ratios (II av. 1.15 and III av. 1.35) serve as satisfactory supplementary evidence to complete the distinction in nearly all cells. The length differences are even

TABLE

COMPARISON OF SOMATIC CHROMOSOME

Species	Mean or S.D.	*Y*-Chromosome		I		II	
		% TCL	Arm ratio*	% TCL	Arm ratio*	% TCL	Arm ratio
Acridiophaga aculeata	M.	14.1	2.92	20.4	5.06	13.4	1.07
	S.D.	—	—	1.081	—	0.530	0.060
	M.	—	—	20.8	3.64	12.9	1.19
Kellymyia kellyi	M.	—	—	10.7	3.76	15.1	1.10
	S.D.	—	—	1.087	0.651	0.665	0.045
	M.	—	—	10.4	6.53	15.7	1.13
Protodexia australis	M.	2.97 (4)	1.19 (3)	10.0	5.00 (4)	16.1	1.08
	S.D.	0.359(4)	0.172(3)	0.485	0.721(4)	0.529	0.067
Protodexia hunteri	M.	3.36 (8)	1.00 (1)	7.2	1.38 (7)	16.6	1.15
	S.D.	0.597(8)	0.000(1)	0.891	0.252(7)	0.714	0.073
Sarcophaga aldrichi	M.	3.5	—	6.7	1.55 (8)	16.6	1.10
	S.D.	—	—	0.524	0.155(8)	0.790	0.067
	M.	2.7	—	6.5	1.55	16.8	1.09
	M.	—	—	6.4	—	16.5	1.12
Sarcophaga cooleyi	M.	—	—	8.9	1.10(1)	16.8	1.12
	S.D.	—	—	0.498	0.00(1)	0.415	0.073
Sarcophaga "H"	M.	5.86 (7)	—	9.0	1.21 (7)	14.8	1.09
	S.D.	0.571(7)	—	0.559	0.448(7)	0.765	0.040
Sarcophaga reversa	M.	4.76	1.22(2)	8.8	1.12 (5)	15.1	1.07
	S.D.	0.552	0.035(2)	0.436	0.106(5)	0.541	0.038

**Secondary arm ratios.*

NOTE: *Numbers in parenthesis show the number of Y-chromosomes, or of pairs of X-*

less conspicuous for Pairs III and IV, but here again the arm ratios for the two pairs are helpful. Pairs IV, V, and VI are separable on the basis of percentage differences alone. There is some difficulty in recognizing the chromosome pairs of these complements.

A secondary constriction was found near the middle of the short arm of Chromosome VI and occasionally one was seen near the distal end of the same arm. In four cells a subterminal secondary constriction was seen near the distal end of the longer arm of this chromosome. A secondary constriction divides the *X*-chromosome which is telocentric, or nearly so, into two parts,

III

COMPLEMENTS IN THE SARCOPHAGIDAE'

Chromosome pairs								Av. arm ratios II–IV	Av. TCL in μ	No. of cells
III		IV		V		VI				
% TCL	Arm ratio	% TCL	Arm ratio	% TCL	Arm ratio	% TCL	Arm ratio			
14.8	1.34	15.7	1.55	17.5	1.33	18.2	1.28	1.31	70.4	13
0.536	0.105	0.558	0.080	0.749	0.154	0.597	0.104			
14.6	1.25	16.0	1.47	16.8	1.47	18.9	1.29	1.33	56.6	4
16.1	1.31	17.1	1.51	19.8	1.16	21.2	1.75	1.37	74.6	15
0.542	0.142	0.955	0.114	0.895	0.073	0.886	0.115			
16.8	1.30	17.6	1.44	19.0	1.14	20.5	1.75	1.35	88.2	3
16.7	1.36	17.3	1.56	18.5	1.56	21.5	1.17	1.34	60.0	6
0.410	0.193	0.219	0.149	0.663	0.151	1.235	0.085			
17.5	1.35	18.0	1.58	19.2	1.32	21.5	1.34	1.35	64.1	12
0.501	0.118	0.830	0.121	0.406	0.151	0.878	0.057			
17.4	1.48	18.7	1.56	19.8	1.17	20.8	1.38	1.34	48.0	11
0.978	0.168	0.402	0.121	0.699	0.086	0.876	0.164			
17.9	1.53	18.7	1.60	19.3	1.20	20.8	1.50	1.37	56.8	3
18.5	1.50	18.5	1.50	19.1	1.14	21.1	1.30	1.31	49.3	1
17.1	1.98	17.7	2.22	18.7	1.37	20.9	1.41	1.62	64.9	5
0.365	0.113	0.705	0.064	0.377	0.054	1.272	0.082			
17.0	1.51	18.1	1.68	20.2	1.39	20.9	1.37	1.41	76.7	17
0.619	0.098	0.804	0.100	0.720	0.101	0.968	0.069			
17.0	1.61	18.6	1.84	19.6	1.35	20.9	1.45	1.47	75.7	9
0.490	0.111	0.439	0.105	0.557	0.055	0.566	0.077			

chromosomes, used to obtain the statistic.

so that the longer segment averaged 1.38 times the shorter, presumably proximal, segment. Another secondary constriction is located near the distal end of the longer segment mentioned above.

The *Y*-chromosomes from seven cells averaged 47.7% of the *X* in length. They are acrocentric and stain very lightly. They sometimes look bipartite and the constriction is interpreted as a secondary one on the basis of anaphase appearance. The *X* appears to be heteropycnotic in metabolic nuclei but is not noticeably different from the other chromosomes in staining reaction at metaphase.

Sarcophaga aldrichi Park.

Many larvae and early pupae of this parasite, reared from the spruce budworm, *Choristoneura fumiferana*, in 1949, were available but few really good preparations were obtained and, in general, analysis was difficult. The 11 cells analyzed came from four females and one male, but in addition several complements from other males were drawn and partly analyzed. In all cases the somatic complements consisted of 12 chromosomes. They varied from 37.6 μ to 70.2 μ in TCL with 48.0 μ as the average and the results of their analysis are presented in Table III and Text-fig. 6. The chromosome complement is shown in Plate I, Fig. 8.

There was considerable overlapping of the ranges of both percentages of TCL and arm ratios for pairs of similar length. Pair I (the *X*-chromosomes) was distinct in length. Pair II was usually shorter than III and distinctly lower in arm ratio in nearly all cells. It is fortunate that Pair III is usually distinctly shorter than Pair IV since their arm ratios are very similar (1.48 for III and 1.56 for IV). The differences in arm ratio usually suffice to separate Pairs IV and V, whereas Pairs V and VI are usually distinct in length. The low average of arm ratios for Pairs II–VI (1.34) places this species in a low arm-ratio category.

Secondary constrictions are common in the autosomes and complicate identification of the kinetochore in certain cases. Long arms of seven Pair II chromosomes had proximal segments averaging 0.87 of the distal ones in length; short arms of three of them had proximal segments averaging 1.76 times the distal. Proximal to distal ratios of segments in the arms of other autosomal pairs averaged as follows: the long arm of III, 0.51 for six chromosomes and 0.90 for three; the short arm of III, 0.49 for five; the long arm of IV, 0.89 for 17; the long arm of V, 0.50 for five and 0.89 for three; the short arm of V, 0.99 for four; the long arm of VI, 0.27 for four, 0.90 for nine, and 1.87 for five; the short arm of VI, 1.82 for five. The secondary constrictions of the long arms of Pair IV chromosomes are the most conspicuous and useful.

The acrocentric *Y*-chromosome is very small (corresponding to 3.5% of TCL and 52.2% of *X*) and stains more lightly than the *X*, which resembles the autosomes at metaphase in staining reaction. Secondary constrictions were found in seven *X*-chromosomes (secondary arm ratios averaged 1.55) but no secondary constrictions were found in *Y*-chromosomes. Heteropycnosis is not obvious, if present.

Several larvae which had been reared on artificial medium were obtained in 1951. Single complements from three larvae gave average results as shown in Table III. These complements averaged 56.8 μ in total length. Also, a single complement (49.2 μ in total length) from another larva taken from a tent caterpillar, *Malacosoma* sp., in the summer of 1952, gave results also shown in Table III. These results are in close agreement with those recorded for the first collection and hence they do not suggest that the later collections represent distinct subgroups of this species.

Sarcophaga cooleyi (Ald.)

Five somatic complements, from two presumably female larvae which had been reared on pork liver, were analyzed. Every complement contained 12 chromosomes and they averaged 64.9 μ in total length with a range from 56.2 μ to 79.3 μ. Results of analyses are recorded in Table III and the complement is illustrated in Text-fig. 7 and Plate I, Fig. 10.

The acrocentric chromosomes of Pair I are distinct in size and stain like the autosomes at metaphase. Pairs II and III are similar in length but II

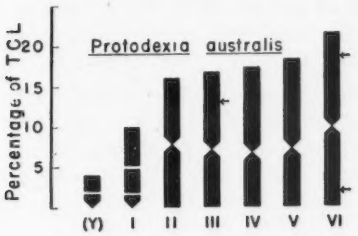


Figure 4

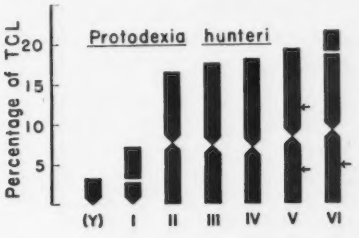


Figure 5

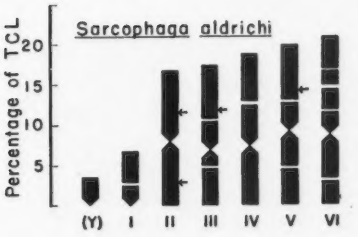


Figure 6

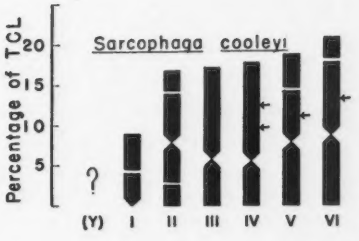


Figure 7

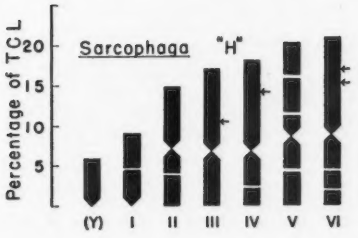


Figure 8

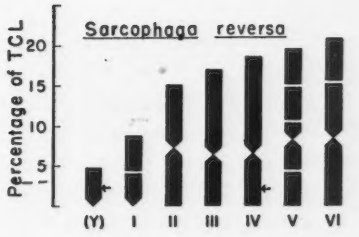


Figure 9

TEXT-FIGS. 4–9. Idiograms of somatic chromosome complements.

averaged 1.12 and III averaged 1.98 in arm ratio. Pair IV has a still higher arm ratio (2.22) but is only slightly longer than III. Pairs V and VI have both much lower arm ratios than IV and may be distinguished from each other by the greater length of VI and the differences in the positions of secondary constrictions. The average of arm ratios of Pairs II–VI is unusually high, 1.62.

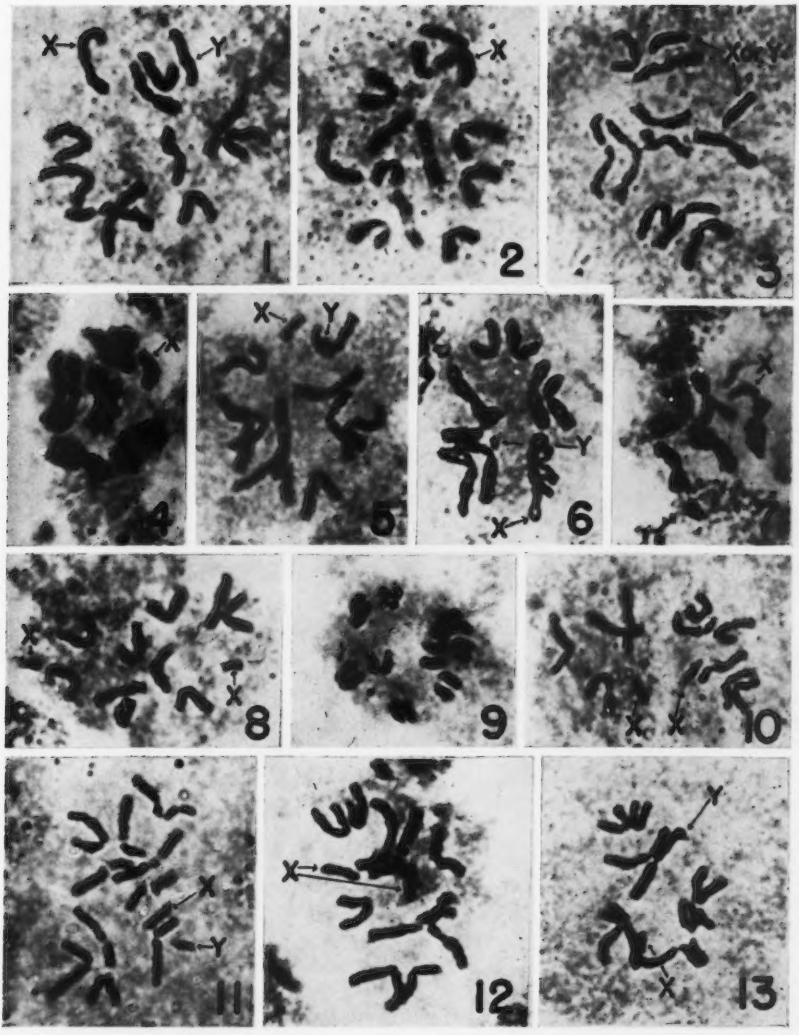
Many secondary constrictions were found. Proximal to distal ratios of segments for the arms of the chromosome pairs averaged as follows: the long arm of II, 2.04 for four chromosomes; the short arm of II, 2.02 for four; the long arm of IV, 0.51 for three and 1.30 for three; the long arm of V, 1.41 for five and 0.38 for two; the long arm of VI, 3.11 for five and 0.58 for three. The constrictions in Pairs V and VI are usually more distinct than others and are useful in separating these two pairs. The two larvae used were apparently females since the sex chromosomes did not differ in any complement. Heteropycnotic regions are detectable in the metabolic nuclei and probably represent the sex chromosomes or parts of them.

Sarcophaga "H"

Larvae of this species have been tentatively designated by the letter "H" by Smith and Finlayson (8) until they can be associated with their adult stages or associated with adults of an undescribed species. They resemble larvae of *Sarcophaga reversa*. Seventeen complements from seven larvae reared on grasshoppers, *Melanoplus* sp., were analyzed. The total lengths of the complements ranged from 63.2 μ to 88.7 μ, and averaged 76.7 μ. Results of analysis are presented in Table III and the complement illustrated in Text-fig. 8 and Plate I, Figs. 11 and 12.

The acrocentric chromosomes of Pair I constitute 9% of the TCL. Pair II is considerably longer and also distinct from Pair I in arm ratio. Separation of Pairs III and IV is more difficult and less certain but Pairs IV and V are usually separable on the bases of length and arm ratio differences. Also, Pair V chromosomes have distinct secondary constrictions in both arms as stated in the next paragraph. Since Pairs V and VI are very similar in length and in arm ratio, they are only separable on the basis of position and distinctness of the secondary constrictions. Arm ratios of Pairs II–VI averaged 1.41.

Secondary constrictions produced proximal to distal ratios of segments in the chromosome pairs, which averaged as follows: the short arm of II, 0.81 for seven chromosomes; the long arm of III, 0.56 for four; the long arm of IV, 1.82 for four; the short arm of IV, 2.19 for six; the long arm of V, 0.31 for 20 and 1.71 for eight; the short arm of V, 1.00 for 24; the long arm of VI, 1.05 for four and 1.97 for four; the short arm of VI, 1.11 for eight and 3.95 for five. Other constrictions were occasionally noticed. Since there are four distinct constrictions in Pair V chromosomes, it is difficult to decide which one is the kinetochore. A study of the association of homologues at metaphase and of their appearance at anaphase helps in reaching a decision.



FIGS. 1–13. Somatic chromosome complements of sarcophagid species at metaphase in brain cells, ×1800. Sexes indicated have not been confirmed morphologically. FIG. 1. *Acridiophaga aculeata*, male. FIG. 2. *Acridiophaga aculeata*, female. FIG. 3. *Kellymyia kellyi*. FIG. 4. *Kellymyia kellyi*, one sex chromosome. FIG. 5. *Protodexia hunteri*, male. FIG. 6. *Protodexia australis*, male. FIG. 7. *Protodexia australis*, female. FIG. 8. *Sarcophaga aldrichi*, female. FIG. 9. *Pseudosarcophaga affinis*. FIG. 10. *Sarcophaga cooleyi*, female. FIG. 11. *Sarcophaga* "H", male. FIG. 12. *Sarcophaga* "H", female. FIG. 13. *Sarcophaga reversa*, male, tiny *Y* at end of an autosome.

The seven larvae apparently consisted of three males and four females. The presumed *X*- and *Y*-chromosomes are acrocentric and stain like the autosomes at metaphase. The *Y*-chromosomes averaged 65% of the *X*, corresponding to 5.9% of the TCL, and no secondary constrictions were found in them. Heteropycnotic regions in the metabolic nuclei probably represent these sex chromosomes.

Sarcophaga reversa Ald.

Nine complements from three larvae reared on grasshoppers, *Melanoplus* sp., were analyzed in 1952. All complements consisted of 12 chromosomes and ranged in TCL from 62.8 μ to 92.4 μ with 75.7 μ as the average. Results of analysis are summarized in Table III and illustrated in Text-fig. 9 and Plate I, Fig. 13.

Chromosomes of Pair I are distinct in size. Pairs II and III differ greatly in length and arm ratio. Pair IV has a high arm ratio (1.83) differing considerably from that of III (1.61) and V (1.35) and differs in most cases from III and V in length also. Pairs V and VI are similar in arm ratio and percentage of TCL and secondary constrictions are helpful in separating them. Arm ratios of Pairs II–VI averaged 1.47.

Few secondary constrictions were found in chromosomes of this species. Proximal to distal ratios of arm segments in the pairs averaged as follows: the short arm of IV, 2.23 for three chromosomes; the long arm of V, 0.24 for 13 and 1.42 for seven; the short arm of V, 0.91 for five; the long arm of VI, 1.28 for four. The constrictions in Pair V are quite distinct and, since they can be seen in most chromosomes, they are useful in identifying this pair.

All three of the larvae used were apparently males since chromosomes of the small acrocentric pair were regularly heteromorphic. The presumed *Y*-chromosome averaged 55% of the *X* and corresponded to 4.8% of the TCL. Secondary constrictions divided two of the *Y*-chromosomes so as to produce secondary ratios averaging 1.22. The sex chromosomes stained like the autosomes at metaphase and appeared to be heteropycnotic in metabolic nuclei.

Comparison of Complements of Sarcophagid Species

The 19- or 20-chromosome complement of *Pseudosarcophaga affinis* is unique in number and chromosome morphology among the sarcophagid species studied to date. The results of the analyses of the 12-chromosome species have been assembled in Table III.

The complement of *Acridiophaga aculeata* can be distinguished from those of the other species listed in the table by its conspicuous *X*- and *Y*-chromosomes. The heterochromatic Pair I of *Kellymyia kellyi* is somewhat larger than that in the remaining 12-chromosome species and has an unusually high secondary arm ratio (3.76). Hence, these two species may be separated from the other 12-chromosome species through recognition of Pair I.

The two *Protodexia* species have similar complements but that of *australis* has a larger Pair I with a lower secondary arm ratio and the arm ratios for Pairs V and VI also differ. The four *Sarcophaga* species have a variety of differences between their complements. Pair I of *aldrichi* is appreciably smaller than in the other three and, where it has been seen, the *Y*-chromosome is smaller than in the other two species. Arm ratios of the four largest autosomal pairs are relatively low in *aldrichi* so that the average arm ratios of Pairs II–VI are relatively low. On the other hand, arm ratios of the autosomal pairs are relatively high in complements of *cooleyi*. The complements of *reversa* and "H" are very similar, but, in *reversa*, the *Y*-chromosome is smaller, Pair IV has a higher arm ratio (1.83 vs. 1.68 for "H") and the proximal segment of the long arm of Pair V seems shorter. These small differences require confirmation but indicate that the complements of *reversa* and "H" differ. The differences between the complements of *P. hunteri* and *S. aldrichi* are not very great. Pair I of *aldrichi* is smaller and has a higher secondary arm ratio, its Pair III has a higher, and its Pair V a lower, arm ratio than in complements of *hunteri*. Secondary constrictions in Pairs II, III, IV, and VI of *aldrichi*, not found in *hunteri*, help to distinguish complements of these two species.

The differences cited above should suffice for the separation of most of these species complements. In general the presumed sex chromosomes show the greatest differences between species, varying greatly in both staining reaction and size. The autosomes, particularly Pair IV, also show appreciable variation between complements in both arm ratio and the position of secondary constrictions.

Discussion

The present paper adds descriptions of the somatic complements of nine species to the few recorded in the literature. As mentioned in the first paper of this series, Stevens (9) described the somatic complement of *Sarcophaga sarraciniae*, Metz (5, 6) studied six sarcophagid species, and Keuneke (4) described the somatic complement of *Sarcophaga carnaria* L. With only one exception, all species possess 12 chromosomes. The exception is the complement of *Pseudosarcophaga affinis*, which differs strikingly from those of other species of this family. The only comparable complement in the higher Diptera known to the writer is that of *Melophagus ovinus* which according to Cooper (3) has 18 chromosomes consisting of eight large autosomes, eight small autosomes, and an unequal XY pair.

One might postulate the origin of the *P. affinis* complement from the 12-chromosome complement of other species through the breakdown at the kinetochore of four metacentric pairs to produce 16 telocentric chromosomes, Even so, the *affinis* chromosomes still differ in respect to their small size and secondary constrictions. Thus it seems that *P. affinis* is an old, well-established type of doubtful relationship to species of the Tachinidae or Sarcophagidae studied to date. Perhaps a reconsideration of its taxonomic position is indicated.

An attempt was made to analyze somatic complements of species of Sarcophagidae and Calliphoridae figured by the authors mentioned above and by Naville (7). In both families it was found that important differences exist between the complements of most of the species figured. For example, in Metz' (5) Figs. 98 and 99 of *Ravinia peninculata* the sex chromosomes averaged 5.8% of the TCL whereas in his Figs. 87 and 88 of *Sarcophaga tuberosa serraceniae* the sex chromosomes correspondingly averaged 8.7%, although Metz from his general comparisons of the chromosomes of *R. peniculata* considered "the six pairs of chromosomes essentially like those of Sarcophaga".

Differences between complements of sarcophagid species are of nearly the same general order as those found in the Tachinidae by Boyes and Wilkes (2). Both families have species with high and with low average arm ratios for Pairs II–VI. Since grouping on this basis would separate species in the genus *Sarcophaga*, as noted below, it would seem that these differences are not as fundamental as they appear to be in the Tachinidae. Pair I of *Acridiophaga aculeata* (20.4% of TCL) approaches the proportions of Pair I of *Winthemia datanae* (31.0% of TCL), although they differ in position of the kinetochore, whereas Pair I of *Sarcophaga aldrichi* (6.7% of TCL) is nearly as small as that of *Neophorocera hamata* (4.8% of TCL). Complements of the sarcophagid species differ more in the frequency and position of secondary constrictions.

Even within the genus *Sarcophaga* there are appreciable differences between species complements. Analysis of Stevens' (9) Figs. 35 and 36 indicates that in *S. sarraciniae* Pair I averages about 4.9 in percentage of TCL as compared to 9.0 for *Sarcophaga* "H" in the present report. Differences also exist in the positions of secondary constrictions in the autosomes and average arm ratios for these pairs vary from 1.62 for *S. cooleyi* to 1.34 for *S. aldrichi*.

Some consideration should be given to common differences between complements of species of Tachinidae and those of the Sarcophagidae. The great variation existing among complements of both families has been mentioned above and makes the search for common features more difficult. In general, secondary constrictions are more plentiful and in certain chromosomes more distinct in sarcophagid complements consisting of 12 chromosomes. Distinct secondary constrictions were found in certain tachinids, as in Pair VI of *Ceromasia auricaudata*, but they were as a rule less distinct than certain ones in Pairs IV, V, or VI of the various species of Sarcophagidae. Aside from this, no common feature was noted and even this point needs more extensive confirmation.

Acknowledgments

It is a pleasure to acknowledge the co-operation of officers of the Laboratory of Entomology, Belleville, Ont. Mr. R. W. Smith and Dr. H. Coppel provided larvae from their rearing stocks; Mr. A. Arthur supplied larvae of *Sarcophaga aldrichi* and Miss E. Montieth, larvae of *Sarcophaga cooleyi*. Their assistance in this way merits my sincere thanks and appreciation.

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A CYTOLOGICAL AND CYTOCHEMICAL STUDY OF THE SHELL GLAND OF THE DOMESTIC HEN[1,2]

By D. J. McCallion

Abstract

A cytological and cytochemical study has been made of the oviducts of hens producing thick shelled eggs and of hens producing thin shelled eggs, with special reference to the shell gland. No significant differences between them were discovered. The inner surface of the oviduct of the hen is lined with ciliated epithelium containing two types of cells, ciliated and goblet. Lying under the ciliated lining are the tubular glands of the oviduct. The Golgi apparatus has been described in the several types of cells. The secretory products of these cells have been identified in the various parts of the oviduct. Small amounts of calcium were shown to be present in the oviduct by one of several techniques, the others giving negative results. Slight positive results were obtained for the presence of phosphates. Alkaline phosphatase was present in slight and varying amounts uniformly distributed in the oviduct, but acid phosphatase was confined to the ciliated epithelium. Glycogen was not present in the glandular tissues. The deposition of calcium as egg shell seems to be different from that in bone formation. Possible mechanisms of egg shell formation are discussed.

Introduction

This development of the embryo within the hen's egg has been extensively investigated, but relatively little attention has been given to the processes by which the egg itself is formed. Interest in the biochemistry and physiology of egg formation has only recently developed. In view of a large poultry and egg industry, the problems involved in egg formation are of considerable importance.

Many important commercial properties of the hen's egg are directly or indirectly related to the quality of the shell. Among these are the interior quality of the egg, storage qualities, hatchability of the egg, and losses incurred by the breakage of thin shells in grading and shipping. It is desirable, therefore, to attempt to determine what mechanisms in the shell gland (uterus) are involved in egg shell formation.

It is already well known that many external factors influence the quality of the egg shell. Variations in environmental temperature, humidity, and pressure are reflected in variations in the calcium level of the blood of the hen, and hence, in the quality of the egg shell. Other factors involved are dietary calcium intake, altered activity of the endocrine organs of the hen, and the position of the egg in the clutch. However, even under suitable environmental conditions with a high level of calcium intake some hens habitually lay thin shelled eggs while others lay thick shelled eggs. This paper presents the results of histochemical and cytological studies of the shell glands of hens laying heavy-shelled eggs and of hens laying thin-shelled eggs. Studies of other portions of the oviduct of the hen have been made for comparison.

[1] *Manuscript received August 13, 1953.*
Contribution from the Perry Biological Laboratories, Acadia University, Wolfville, N.S.
[2] *This investigation was supported by the Nova Scotia Research Foundation.*

Materials and Methods

The birds used in this investigation were healthy, mature, actively laying white leghorn hens from the flock maintained at the Dominion Experimental Station at Kentville, N.S. The thickness of the shells of the eggs laid by these hens was measured by means of a penetrometer developed by Mr. Arthur Kelsall of the Experimental Station Staff. The penetrometer recorded the pressure in grams necessary to cause a needle to penetrate the shell at the end of the egg. Ten hens were selected as producing heavy-shelled eggs with an average shell strength of 1294 gm. Ten hens were selected as producing thin-shelled eggs with an average shell strength of 672 gm. Two hens laying shell-less eggs were also included.

At the time that the birds were killed, a fully formed egg with incompletely calcified shell was present in the uterus. The birds were sacrificed by severing the blood vessels of the neck. Within a few minutes the oviduct was dissected out and small blocks of tissue from the shell gland were placed in suitable fixing solutions. At the same time, for purposes of comparison, small blocks of tissue from the albumen-secreting portion and the isthmus were similarly fixed. Unless otherwise stated the tissues were dehydrated through dioxane and embedded in paraffin.

1. For routine histological examination tissues were fixed in Bouin solution and stained with Delafield's hematoxylin and eosin. Bouin fixed material was also stained with mucicarmine.

2. The DaFano cobalt nitrate – silver method was used for the study of the Golgi apparatus. Tissues were fixed in cobalt nitrate solution for six to eight hours, washed in distilled water, and impregnated in 1.5% aqueous silver nitrate. The tissues were then reduced in hydroquinone mixture for 24 hr.

3. For studies of the distribution of alkaline and acid phophatases, tissues were fixed in chilled acetone, dehydrated in acetone, and cleared in benzene. Paraffin sections were incubated for 12–24 hr. in solutions of glycerophosphate, buffered to pH 4.5 for acid phosphatase, and pH 9.4 for alkaline phosphatase. Sites of phophatase activity were rendered visible by converting the phosphate liberated to black lead sulphide in the case of acid phophatase and to cobalt sulphide in the case of alkaline phophatase.

4. For the detection of phosphates, tissues were fixed in acetic–alcohol–formalin solution and washed in water. In order to hydrolyze organic phosphates and to precipitate the free phosphates, the tissues were immersed in a solution of ammonium molybdate and hydrogen chloride for two weeks or longer. The tissues were then treated with acetic benzidine solution and mounted in glycerol saturated with sodium acetate.

5. Tissues fixed in 10% formalin were used in several techniques.

(*a*) Von Kóssa's silver method for calcium, in which sections were saturated in 5% silver nitrate solution in the dark for one hour and exposed to bright light.

(*b*) The Cretin color test for calcium, in which sections were exposed to a mixture of metaformaldehyde, gallic acid, and ammonium hydroxide.

(*c*) Tests with sulphuric acid and oxalic acid for the formation of characteristic crystals of calcium.

(*d*) Tests for manganese using the ferrocyanide reaction.

(*e*) Sections were stained with mucicarmine.

6. Tissues fixed in chilled picro–alcohol–formalin were treated with the periodic acid – Schiff reagent for the presence of glycogen. One set of companion sections was incubated in saliva or buffered malt diastase prior to treatment. A second set of companion sections was incubated in a 2% solution of sodium bisulphite in order to bind any free tissue aldehydes.

Results

The organs involved in egg formation are the ovary and the oviduct. The germ cells and the yolk of the egg are produced in the ovary. The albumen, two shell membranes, and the shell are added to the egg as it passes down the oviduct. The oviduct of the hen is a single, long, convoluted, tubular organ with elastic walls and of considerable but varying length. The oviduct of the hen has been divided by Pearl and Curtis (37) into five morpholigical divisions, each having specific functions in the formation of the egg. These regions are: (1) the infundibulum or funnel, (2) the magnum or albumen-secreting region, (3) the isthmus or shell membrane-secreting region, (4) the uterus or shell-secreting region and (5) the vagina. The tissues of greatest importance in the oviduct are the muscle layers which propel the egg through the oviduct and the glandular epithelium lining the oviduct. It is with the latter that the present investigation is concerned.

The histology of the oviduct of the hen has been described by Surface (44) and extended by Bradley (4). The epithelium lining the oviduct is much folded and wrinkled. The lining of the oviduct consists of two parts: (1) an inner ciliated epithelium which contains unicellular epithelial glands occurring between the ciliated cells, and (2) an outer mucous membrane which forms a thick layer of tubular glands beneath the epithelium. These are long, convoluted, and branched tubules which open into the lumen of the oviduct by short ducts.

The Golgi apparatus of the inner epithelial layer as revealed by the DaFano technique is composed of coarse granules. The nuclei of the nonciliated goblet type cells are located towards the basal end of the cell and the Golgi apparatus is a mass of short rods and granules arranged in a line extending upward for half the length of the cell (Fig. 3). These often have the appearance of a long thread. There are many variations in the position and form of the Golgi apparatus and occasionally granules may be found at the basal pole of the nucleus. The nuclei of the ciliated cells are located towards the distal end of the cell and the Golgi apparatus is usually a dense mass of granules lying between the nucleus and the distal edge of the cell. Occasionally the Golgi apparatus is located at both poles of the nucleus (Fig. 3). The secretory

material of the cells accumulates at the distal edge of the cell and creates artifacts which somewhat confuse the picture of the Golgi material. The amount of argentophile material in the ciliated cells is considerably greater than in the goblet cells. In both types of cell a network configuration was rarely seen.

In the cells of the tubular glands, the Golgi apparatus is composed of threads, rods, and granules. There is considerable variation in the form, amount, and distribution of the Golgi material, but it is usually a typical network (Figs. 1 and 2). In the tubular glands of the albumen-secreting portion of the oviduct and of the isthmus, the cells are packed with large distinct secretory granules and the nucleus is pushed towards the base of the cell. In such cells the Golgi apparatus appears as a compacted close network immediately above the nucleus or between the granules (Fig. 1). In those cells which were exhausted of their secretion or which contained relatively few secretory granules, the Golgi material formed a loose network which occupied the middle region of the cell and was intimately related to the secretory material. In the cells of the tubular glands of the shell gland, the nucleus is large, round, and located in the middle of the cell. No secretory material was demonstrable with silver. The Golgi apparatus formed a loose network between the nucleus and the lumen of the gland (Fig. 2).

Alkaline phosphatase was present in the oviduct of the hen, but in slight and variable amounts. Alkaline phosphatase activity was slight but uniformly distributed in the epithelial lining and in the tubular glands in the albumen-secreting portion, in the isthmus, and in the shell gland. There was dense alkaline phosphatase activity in the walls of the blood vessels of the oviduct. No relationship between the variations in alkaline phosphatase activity and the shell strength of the eggs laid by the hens could be demonstrated.

Acid phosphatase activity was confined to the nuclei of the cells of the tubular glands and to the nuclei and cytoplasm of the cells of the epithelium (Fig. 4). This enzyme was abundant in the nuclei and cytoplasm of the goblet type cell and was present as fine granules, dense in the distal parts of the cells, and less dense in the basal regions. Acid phosphatase activity in the ciliated cells was somewhat less than in the goblet cells. There seemed to be no relationship between acid phosphatase activity and shell strength.

FIG. 1. Photomicrograph of a tubule of the tubular glands of the albumen-secreting region of the oviduct of the hen, showing the Golgi apparatus and secretory droplets. DaFano. ×1125.

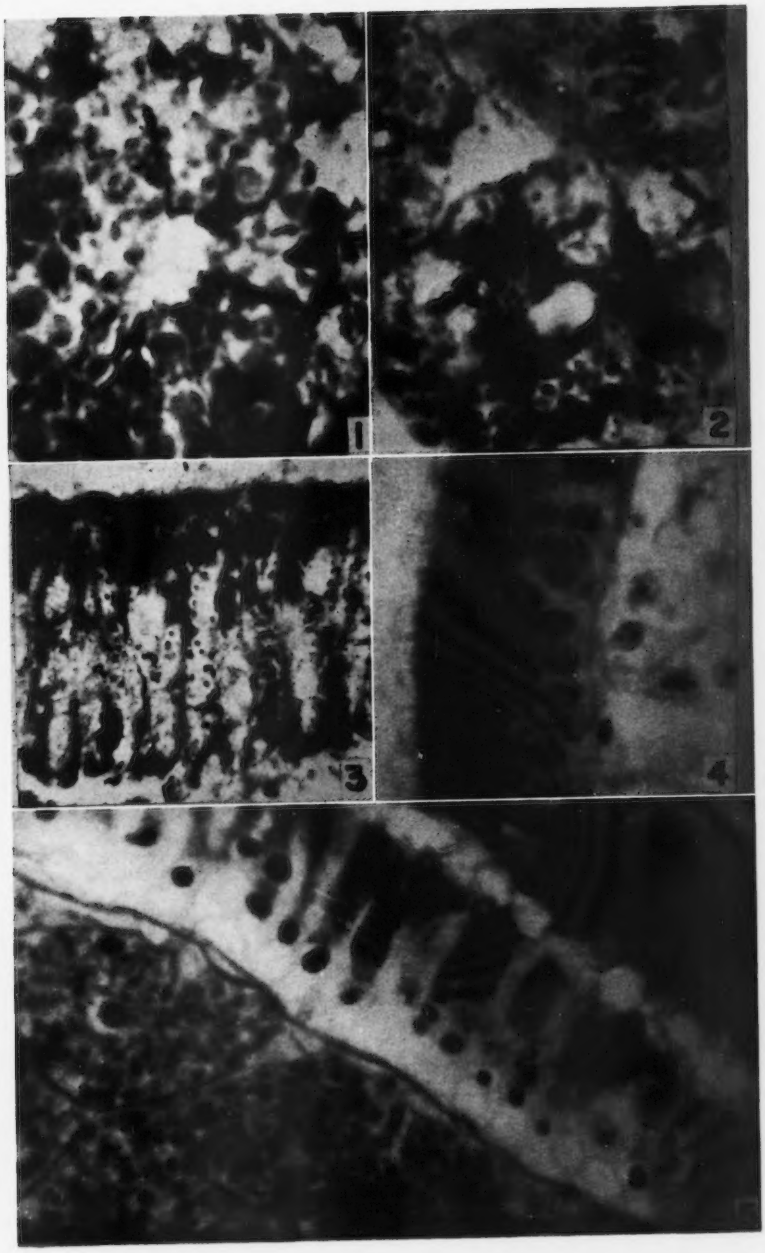
FIG. 2. Photomicrograph of a tubule of the tubular glands of the uterine portion of the oviduct of the hen, showing the Golgi apparatus. DaFano. ×1125.

FIG. 3. Photomicrograph of the ciliated epithelium of the albumen-secreting portion of the oviduct of the hen, showing the Golgi apparatus. DaFano. ×1125.

FIG. 4. Photomicrograph of the albumen-secreting portion of the oviduct of the hen. Acid phosphatase in the ciliated epithelium and in the nuclei of the tubular gland cells. Gomori. ×500.

FIG. 5. Photomicrograph of the albumen-secreting region of the oviduct of the hen. Periodic acid – Schiff reagent. Secretion in the lumen of the oviduct, upper right. Secretory droplets in the tubular gland cells, lower left. Note clear droplets at the distal edge of the goblet cells. ×500.

PLATE I



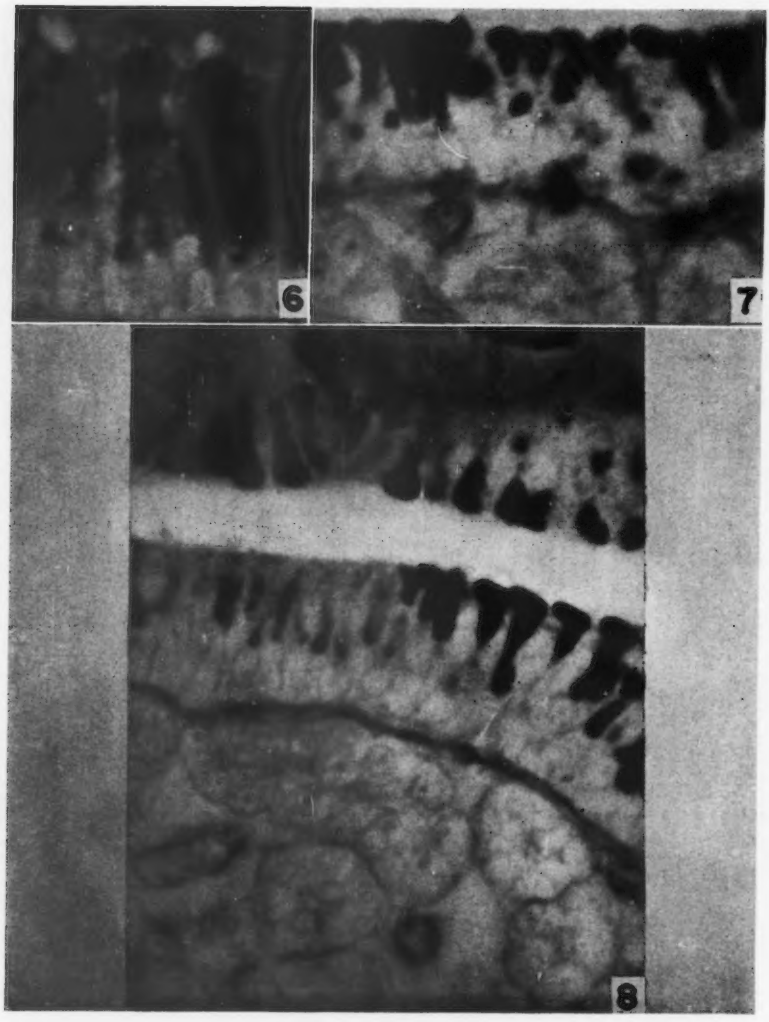


FIG. 6. Higher magnification of the ciliated epithelium shown in Fig. 5. ×1000.
FIGS. 7 and 8. Photomicrographs of the uterine portion of the hen's oviduct. Periodic acid – Schiff reagent. Compare Figs. 5 and 6. Note absence of stain in the tubular gland cells and different character of staining of the goblet cells. ×500.

Tests for the presence of phosphates gave only slight positive results. The technique used to demonstrate the presence of phosphorus in tissues depends upon the precipitation of inorganic phosphates and in the liberation of phosphates in organic combination. The amounts of phosphates so demonstrated were slight and uniformly distributed throughout the oviduct. There were no appreciable differences between the albumen-secreting portion and the shell gland of any given oviduct, nor were there any differences between the oviducts of thick shell layers and thin shell layers.

The procedures used in the demonstration of alkaline phosphatase also reveal the presence of insoluble calcium salts. In the detection of alkaline phosphatase, these salts are removed before incubation with the substrate by treatment with a citrate buffer of pH 4.5 to 5.0 for 15 min. In the albumen-secreting portion and in the isthmus, almost negligible amounts of calcium were present, and only slightly greater amounts were present in the shell gland. There were no significant differences between the oviducts of thick shell layers and thin shell layers. The calcium was not localized in these tissues, but was uniformly distributed. Silver nitrate is a well-known but unreliable reagent for the identification of calcium in tissues. The Von Kóssa method of demonstrating the presence of calcium gave entirely negative results. The Cretin color reaction is cited by Lison (32) as extremely sensitive for calcium and having a high degree of specificity. With this test, calcium gives a blue color. No reaction was obtained in the upper regions of the oviduct, but very slight positive results were obtained in the uterus. Treatment with sulphuric acid or oxalic acid was not sufficiently sensitive to produce the characteristic crystals of the calcium salts of these acids.

In the hen, manganese contributes indirectly to the formation of egg shells of adequate thickness (Romanoff and Romanoff (40)). Tests for the presence of manganese using the ferrocyanide reaction gave entirely negative results. Manganese, if present, was not present in amounts sufficiently great to be detected by this procedure.

Tissues treated with the periodic acid – Schiff reagent gave very significant results. The tissues of the oviduct retained their ability to recolor the Schiff reagent after digestion in saliva or in buffered malt diastase and, therefore, the staining reaction was not due to glycogen. Similarly, the staining ability of these tissues remained after treatment with sodium bisulphite solution and, therefore, the staining reaction was not due to the presence of free or loosely bound tissue aldehydes.

In the epithelium of the albumen-secreting region and of the isthmus the basement membrane was deeply stained. The ciliated cells were entirely unstained. In the goblet cells there were large thready masses which stained deeply purple (Figs. 5 and 6). These masses seemed to be composed of large granules. The distal edges of these cells were not discernible. The secretion material appears to lose its staining capacity when it leaves the cell and large clear droplets can be seen beyond the deeply stained contents of the cell (Fig. 5). Frequently a vacuole with a large droplet of deeply stained

material can be seen immediately above the nucleus (Fig. 5). In the lumen of the oviduct, large masses of material are stained. This is probably a mixture of the secretions of the goblet cells and of the tubular glands and demonstrates that these cells are in active secretion sometime after the egg has passed to a lower level of the oviduct.

The lumina of the tubular glands may be filled and distended with deeply stained lakes of secretion, and there may even be extravasation of secretory material between the tubules. The nuclei of the tubular gland cells are not discernible, except in cells relatively free of secretion droplets, when they are large and round. The cells of the tubular glands are usually packed with secretion material as deep purple droplets (Fig. 5). There is a considerable variation in the number of droplets in the cells. When they are few in number they are small and lightly stained. In packed cells the droplets vary from small lightly stained spheres to larger more deeply stained spheres.

The shell gland presents a somewhat different appearance. The basement membrane of the epithelium, however, was also deeply stained. The ciliated cells were entirely unstained. The goblet cells were in various phases of secretion (Figs. 7 and 8). Some cells were packed with a condensed mass of deeply stained granules which appear as a solid mass. At the distal edge of the cell there is a large constricted knob or bleb of deeply stained secretion which breaks up into scattered granules in the lumen of the oviduct. As the cells are exhausted the nuclei move up behind the secretion mass as it is extruded from the cell. When the cells are empty the nucleus appears again towards the base of the cell. Other cells have granular masses reforming in the middle regions of the cell. The cells of the tubular glands of the uterus were almost unstained (Figs. 7 and 8). The nuclei are large, round, and located in the middle of the cells. There is a fine haze of very small and very lightly staining granules between the nucleus and the lumen of the tubule.

Mucicarmine after Bouin's fluid of 10% formalin fixation did not give a test for mucus in any of these tissues. These are considered good fixatives for mucus and Lillie (31) suggests any acid fixative including formalin if it has not been neutralized.

Discussion

In view of Brambell's (5) contention that secretory processes in the columnar cells of the ciliated oviducal epithelium of the hen possibly involve the extrusion of Golgi material from the apical cell cytoplasm followed by its reformation basally, Richardson (39) made a study of both silver and osmium impregnations of the uterine epithelium. He found it impossible to confirm Brambell's contention. Bowen (3) had already pointed out that Brambell's observations were at variance with well-established facts in other secretory cycles. The appearance of argentophile deposits on the ciliated cell membrane and among the cilia described by Brambell were also described by Richardson and are evident in the present study (Fig. 3). As Richardson has pointed out, it is to be expected that any silver impregnation of the oviducal epithelium

will contain artifacts in the lumen which form in association with secretion and cell debris. Richardson (39) also states that the high concentration of calcium in the uterus increases the conditions favorable to the formation of artifacts, but he, himself, was unable to demonstrate any appreciable amounts of calcium by microincineration techniques. In fact it is very difficult to demonstrate calcium in large amounts by any of several histochemical methods, and the calcium content of the oviduct is very low (Buckner, Martin, and Peter (6) and Common (9)). There is no doubt that the impregnated material at the apical edge of these cells is an artifact but this is not due to a high concentration of calcium.

Brambell (5) described the Golgi apparatus of the cells of the tubular glands, following the DaFano method, as reticulate and always lying between the nucleus and the lumen of the tubule. The present material was identical with that of Brambell. The description of the Golgi apparatus in the ciliated oviducal epithelium presented in this paper is essentially the same as that of Richardson (39) except that the large reticulum or network that he described was rarely seen. In general, these facts are typical of ciliated epithelium (Kopsch (25), Ludford (33)). There is no doubt that, as in other types of glands, the Golgi material has some relation to the various secretions of the oviduct, but the excessive activity of this organ makes it impossible to clearly distinguish what that relation may be.

Although it has been frequently demonstrated that there is an appreciable amount of glycogen in the hen's egg (Donhoffer (12), Idzumi (23), and Sakuraji (41)), it is surprising that attempts to stain glycogen in the oviduct with Best's carmine (Richardson (39)) and with the periodic acid – Schiff reagent in the present material were unsuccessful.

Two albumens, ovalbumen and conalbumen, together with two glucoproteins, ovomucin and ovomucoid, are the major constituents of egg white. The glucoproteins obviously will recolor the Schiff reagent following oxidation with periodic acid. Although the nature of the carbohydrate groups in egg albumen are not well known it has been established that some form of polysaccharide constitutes part of the egg albumens (14, 29, 30, 36, 42) and, therefore, they too may be detected by the periodic acid – Schiff reagent. Several attempts have been made to localize the secretion of the substances that constitute the egg white in definite regions of the oviduct. In general, the secretion of protein occurs in the albumen-secreting portion, that of the shell membranes in the isthmus, and that of mineral chiefly in the uterus. The methods used in localizing these substances have been differential staining (Bradley (4), Conrad and Scott (11)), analyses of isolated parts of the oviduct (Buckner, Martin, and Peter (6)), microincineration (Richardson (39)), and surgical resection of parts of the oviduct (Asmundsen and Jervis (1)).

It has been demonstrated that the goblet cells of the albumen-secreting portion of the oviduct secrete mucin. Bradley (4) and Cole (8) have shown by the use of several stains, but principally with mucicarmine and thionin that the mucin secreted is identical with the mucin fibers of egg white. Although

the present material gave poor results with mucicarmine the results obtained with the periodic acid – Schiff reagent are not at variance with Cole's (8) findings and confirm the production of fiberlike mucin in the goblet cells of this region. Bradley (4) and Giersberg (16) have stated that the material is secreted in the form of droplets which disintegrate immediately into fine granules which lose their staining capacity. Surface (44) described it as pouring out in little streams, and Richardson (39) in the form of strings closely related to the lining epithelium. It is not clear from the present preparations (Figs. 5 and 6) what the form of the secretion is, although I am inclined to agree with Richardson that it is in the form of strings. It is quite clear, however, that the material loses its staining capacity upon leaving the cell (Fig. 5).

In the epithelium of the uterus Bradley (4) was unable to demonstrate mucus cells with thionin or other mucus stains, but got only a pale stain which he attributed to a relative lack of mucin. He was of the opinion that the isthmus and uterus were alike. The present material indicates that the isthmus and the albumen-secreting region are alike but that the uterus is different from them. Richardson (39) has described two types of goblet cells in the uterus which he refers to as basal cells and apical cells. These seem to be different states of activity in the secretory processes of the cells. It has not been possible to distinguish these two types of cell in the present material. Richardson states that "it has never been possible to trace the activity of the basal cells of the entire uterine epithelium as ending in a mucous secretory phase with the formation of bleb cells". However, such a condition has been demonstrated throughout the uterine epithelium in the present material (Fig. 8). Stormont (43) emphasized the fact that tests for mucin were (at that time) nonspecific and nonmicrochemical. Bradley (4) also regretted the lack of a suitable technique, and Richardson (39) in his discussion states that this lack of reliable histochemical technique leaves the localization of ovomucin entirely unsolved. In the present material there is no question that the uterine mucosa produces mucin and that it is different in nature from that produced in the albumen-secreting region. It is probable that ovomucin is secreted by the epithelium of the albumen-secreting region and mucus by the uterine epithelium.

Several authors (Surface (44), Bradley (4), Richardson (39)), principally on the basis of hematoxylin staining, have remarked on the differences in the tubular gland cells of the albumen-secreting and uterine regions of the oviduct with regard to the number, size, and location of granules. The present material, after staining by the periodic acid – Schiff reaction, clearly shows the formation and secretion of large droplets of albumen in the albumen-secreting region. It is also clear that the tubular glands are not composed of mixed cells of albumen and mucous types, but are all of the same type (Fig. 5). Richardson (39) has already shown that there are no cells giving a reaction to mucin staining. Allowing for differences in stages of formation of the droplets they all stain alike with the Schiff reagent.

The cells of the tubular glands of the uterus contain few, small, lightly staining granules, which indicates clearly that virtually no albumen is produced in this region. Albumen is produced, however, in the albumen-secreting portion of the oviduct after the egg has passed to the uterus and flows down toward the egg. These observations are in agreement with analyses of the material added to the egg in the uterus. This material contains very little protein and consists chiefly of a water solution of inorganic salts rich in potassium and bicarbonate ions (Hughes (22), Beadle, Conrad, and Scott (2)).

With regard to the localization of calcium secretion in the shell gland the staining evidence from the use of hematoxylin and silver salts is always negative (Richardson (39)). The evidence from ashing by microincineration shows a heavier ash in the epithelium, but this is due to crowding of the cells rather than a greater calcium content (Richardson (39)). Alizarin Red S also gives negative results (Godfrey (17)). In the present material (all of which was fixed at a time when a partially calcified egg was in the uterus) the alkaline phosphatase technique and the Cretin color reaction gave slight positive results, but showed a uniform distribution of calcium throughout both the epithelium and the tubular glands, and there was no significant difference between the uterus and other parts of the oviduct. Chemical analyses of parts of the oviduct (Buckner, Martin, and Peter (6)) show that the uterus contains no more calcium and phosphorus than other parts of the oviduct, and very little difference between laying and nonlaying hens. In fact the entire oviduct contains very little calcium (Common (9)). There is no evidence that calcium is stored in the uterus prior to egg shell formation. The fact that the alkaline phosphatase content of the uterus is also low (Gutowska, *et al.* (19), Godfrey (17)) and that the slight activity present is uniformly distributed offers no evidence for the localization of calcium secretion and indicates that the uterus excretes rather than mobilizes calcium.

Godfrey (17) obtained negative results for acid phosphatase, but it can be demonstrated in the ciliated epithelium throughout the oviduct (Fig. 4). The acid phosphatase activity of these cells is probably not related to calcium production, but it is reasonable to assume that acid phosphatase is involved in the elaboration of the mucous secretions of these cells (McCallion and Aitken (34)).

The results of a number of investigations leaves no question that the deposition of calcium as egg shell and as bone are quite dissimilar processes. Alkaline phosphatase is present in the oviduct in small and variable amounts and glycogen is not demonstrable. It is almost impossible to demonstrate the presence of calcium. On the other hand alkaline phosphatase activity and glycogen deposition are definitely correlated with calcification in the lower jaw of the developing chick embryo (Gibson and McCallion (15)).

The most striking feature of the present investigation is the absence of any significant differences in the histochemical aspects of the oviducts of hens laying heavy-shelled eggs, thin-shelled eggs, or shell-less eggs. The mechanisms governing the differences in the eggs has not been revealed.

In the formation of the egg shell by the shell gland calcium is deposited as the carbonate. Driggers and Comar (13) have shown that as much as 75% of the calcium of the egg is secured directly from the diet, while the remainder is secured from the calcium reserves of the body. The deposition of calcium carbonate in the egg shell is a significant part of the metabolism of calcium in laying hens but the physiology of the process is not yet fully understood.

The rate of calcium carbonate deposition in the egg bears a constant relationship to the time in hours that the egg spends in the uterus (Warren and Scott (45), Burmester, Scott, and Card (7)). This fact would seem to indicate that there is a mechanism regulating the amount of calcium released by the shell gland, although its nature has not been clearly demonstrated.

A definite variation in the calcium level of the blood during egg production has been demonstrated (Hogben and Hogben (21), Knowles *et al.* (24)). The level of calcium is greatest when no fully formed egg is present in the uterus and least during the time of egg shell formation. Laskowski (26, 27) and Greenberg, *et al.* (18) have shown that the increased calcium in the blood of laying hens is in the form of a complex phosphorus-containing protein. This has raised the question whether the phosphatase enzymes are involved in the separation of calcium from its combination with the protein. There also seems to be a direct relationship between the levels of calcium and of inorganic phosphorus in the blood during the laying period, but is not apparent at other times (Greenberg *et al.* (18)).

The plasma phosphatase at the time of shell deposition is 50% higher and the variations of phosphatase are parallelled by the increased inorganic phosphorus (Laskowski (27, 28) and Peterson and Parrish (38)). Gutowska *et al.* (19), in a study of the alkaline phosphatase activity of the blood of good and poor producers and of good and poor shell producers, found that the phosphatase activity of the blood of good producers showed a ratio to poor producers of 2 : 1, but, when shell strength was compared, the ratio of the blood of thin-shell layers to that of heavy-shell layers was only 0.91. These authors dissected out the oviducts, scraped off the mucosa, and determined its enzymatic activity. They found no significant differences in the phosphatase activity of the oviducts of various hens. This fact has also been demonstrated histochemically in the present material.

According to Gutowska *et al.* (19) a variation of the phosphatase activity is observed in the blood at the time of shell deposition while the activity of the enzyme does not vary significantly in the shell gland. Therefore, the shell gland should be considered as an excretory organ of calcium. It is suggested by these authors that the dissociation of the colloidal form of calcium phosphate occurs in the blood rather than in the reproductive system, and that calcium is transferred directly from the blood through the shell gland to the egg. It is much more likely that the high alkaline phosphatase activity of the blood of laying hens reflects the ability of the hen to withdraw calcium from calcium phosphate reserves of the skeleton, and, at the same time, could account for the rise in inorganic phosphorus in the blood.

Gutowska and Mitchell (20) further suggest that the carbonate radical is obtained or produced by the shell gland. The enzyme, carbonic anhydrase, can catalyze either the deposition or solution of calcium carbonate (Meldrum and Roughton (35)). Common (10) found the content of the carbonic anhydrase in the shell glands of good layers to be twice that of non-layers and felt that this enzyme might play a direct role in the deposition of calcium in the egg shell. Gutowska and Mitchell (20) found no difference in carbonic anhydrase activity of the blood of good layers and non-layers, whereas, the shell glands of good layers of strong shells showed two or three times more activity than the shell glands of poor layers of soft shells. These authors suggest that carbonic anhydrase catalyzes the decomposition of carbonic acid formed from the bicarbonates of the blood and that the carbonate ions are utilized in calcium carbonate deposition. Since a calcium proteinate can ionize freely (Weir and Hastings (46)) calcium ions could diffuse out to the surface of the egg where calcium carbonate is deposited (pH 8.2–9.2) but would not be deposited in the slightly acid (pH 6) shell gland. There is no real evidence for the intervention of carbonic anhydrase in the processes of egg shell formation, and the site of the liberation of calcium from its colloid remains obscure. There is no direct evidence that calcium is liberated in the blood, and since no appreciable amounts of phosphates were demonstrable in the oviduct and since the uterus contains no greater amounts of phosphorus than other parts of the oviduct (Buckner, Martin, and Peter (6)), it is doubtful that it is liberated in the shell gland.

No histochemical test for carbonic anhydrase has been developed. If such a test were available it would contribute considerably to the solution of the problem by revealing the distribution of carbonic anhydrase in the various tissues of the shell gland.

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